THE REDUCTION OF 2-ACETYL-1,3-INDANEDIONE OXIME WITH FORMIC ACID

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Master of Arts

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by

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DEDICATION

To all my people, in no particular order, my Dad, my Mother, Richard, Kay, and Joanne, whom most often we love in silence, but we do love indeed.

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INTRODUCTION

Formic acid is unique among organic acids in that it contains the basic aldehyde structure as well as the carboxyl function. Formic acid, like most aldehydes, gives a positive Tollen's test. This makes the acid a potential reducing agent, and formic acid has been used to reduce a variety of organic compounds. The results of these efforts have been quite varied and somewhat unpredictable.

The primary objective of this investigation was to study the reduction of various oximes using formic acid as the reducing agent. Oximes of various ketones were prepared and refluxed with formic acid in order to gain information about the types of oximes that could be reduced under these conditions. As an aside, several different methods of oxime preparation were tried. This was undertaken in an effort to correlate ketone structure and ease of oxime formation. Also, several separation and identification techniques were investigated in connection with identification of products. Both partition chromatography and thin-layer chromatography were used with some success. Several other techniques, including potentiometric titrations and various extraction methods, proved to be difficult to apply.

Formic acid can be oxidized to two possible products, carbon dioxide and oxalic acid. However, all efforts to prove the existence of either of these failed. The evidence implies that carbon dioxide is definitely not formed and that the reaction is somewhat more complex than originally anticipated.

SUMMARY

This project was undertaken to characterize the reaction which occurs when certain oximes, specifically the oxime of 2-acetyl-1,3-indanedione, are heated at reflux with formic acid. The oxime has been shown to be reduced to the corresponding imine. Formic acid is most often oxidized to carbon dioxide, especially in the presence of certain catalysts. An effort was made to detect and measure any carbon dioxide that was produced in the reaction. Several methods were used as a means to accomplish this, but each one failed to detect any of this gas. The other possibility for the oxidized product was oxalic acid. Determination of oxalic acid in the presence of formic acid proved to be a difficult task. By using the techniques of thin-layer and partition chromatography, some success was obtained. The results of these two techniques showed that very little, if any, oxalic acid was present in the product mixture.

Characterization of the reaction proved to be somewhat more difficult than originally imagined. The problem has not been solved, but some conclusions have been reached. The reaction is possibly complicated by one or several side reactions which are mentioned, but no effort was made to validate them. The best possibility, in terms of experi-

mental results, for complication seems to be a disproportionation reaction between molecules of the oxime to produce two products, an oxidized product and a reduced product.

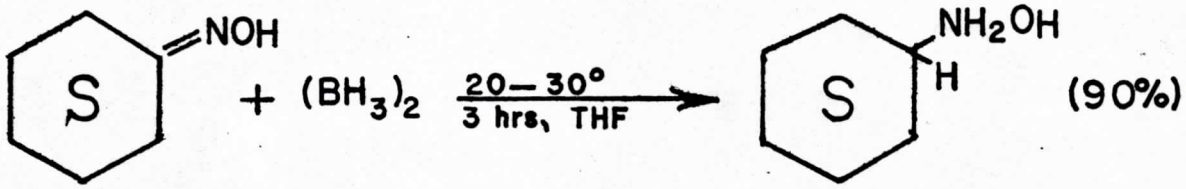
Considerable work was done in finding suitable solvents and adsorbents for use with thin-layer and column chromatography. The various systems tried, along with a summary of results, are included. Another problem proved to be development of the separate bands. In the thin-layer technique, no reagent was found that would discriminate between formic acid and oxalic acid. When a column was used, the problem of monitoring the various bands was solved by impregnating the stationary phase with an acid-base indicator which showed a color change as the acids moved down the column.

HISTORICAL

The reduction of oximes has been studied in some detail, and there are numerous accounts in the literature which discuss various means of accomplishing this. Although these methods are highly diversified, by far the greatest majority involve systems which employ a metal catalyst and H_2. Some of the catalysts which have been used are Raney alloys (Al-Ni), Cu, and ZnO. Other groups of compounds which accomplish this purpose are metal hydrides such as $LiAlH_4$ and non-metal hydrides such as diborane, B_2H_6. C. J. Timmons studied catalytic reductions in relation to selectivity and stereochemical course on a variety of organic compounds.[1] He was able to reduce oximes to N-monosubstituted hydroxylamines with diborane.

It has been shown that improved yields of primary amines can be obtained by reducing nitriles and oximes with Raney alloy and also, oximes can be reduced to amines with Raney-Nickel and sodium hypophosphite.[2] Treatment of ketoximes with Raney-Nickel was shown to yield the corresponding ketone.[2] Refluxing dibenzobicyclo (2.2.2) octadienone oxime in tetrahydrofuran with excess $LiAlH_4$ yielded both anthracene (26%) and aziridine (27%).[3,4]

Solutions of B_2H_6 in tetrahydrofuran have been used to reduce oxo oximes to the corresponding alkylhydroxylamines.[5]



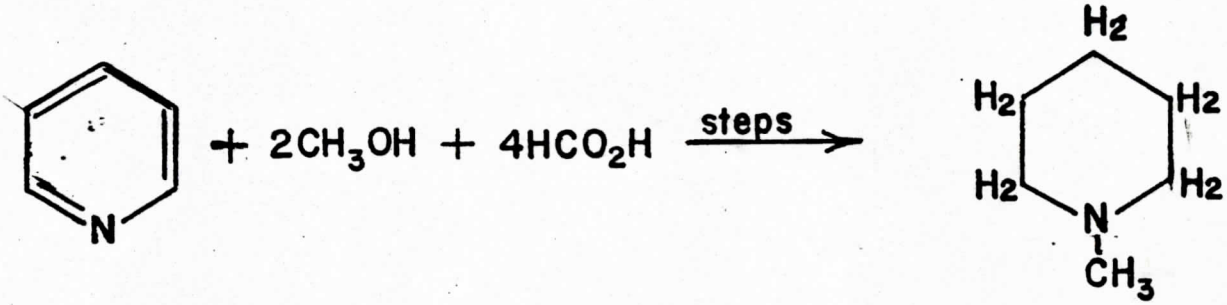
Some work has also been done in the area of electrochemical reductions and oximes can be reduced by this method. An example is the reduction of 4-(4-methoxyphenyl)-1H-2,3-benzoxazin-2-one and $2,4(HO)_2C_6H_3C(:NOH)Ph$ which both showed two two-electron polarographic reduction waves to form the imine $HO_2CC_6H_4C(:NH)C_6H_4(OMe)$-p HCl.[6]

The use of formic acid as a reducing agent has also been used to reduce the oxime function. The most general work has been done by A. N. Kost, L. G. Yudin, et. al.[7,8,9] These workers used formic acid, and derivatives of formic acid, to reduce a number of organic compounds. They reduced quinoline to N-formyl-1,2,3,4,-tetrahydroquinoline (94.4%), for example.

Work has been reported using formic acid as a reducing agent under positive pressure. Reduction by HCO_2H in the presence of catalysts has been effected under pressure and at comparatively low temperatures.[10]

Lauric Acid + HCO_2H + TiO_2 $\xrightarrow[3\ hrs.]{260^{\circ}}$ $C_{11}H_{23}CHO$ (90%).

Mayo, et. al., [11] reported, in 1936, reducing the pyridine ring with formic acid in methanol.



other products (50%)

Simple aliphatic amines are smoothly methylated to the corresponding tertiary amines by warming in HCO_2H with HCHO, the yields being over 80%.[11]

Although the reduction of oximes has not been studied in detail, the reduction of alcohols with formic acid has. Guyot and Kovache [12,13], as early as 1912, found that triphenyl carbinol was almost quantitatively reduced to triphenylmethane in hot concentrated formic acid.

$Ph_3COH \xrightarrow{HCO_2H} Ph_3CH + CO_2$

Later Boroden and Watkins [14] showed that in this reduction the formic ester was first produced and that the triphenylmethane resulted from the decomposition of this ester

$$Ph_3C\text{-}OH \xrightarrow{HCO_2H} Ph_3COCHO \xrightarrow[\Delta]{HCO_2H} Ph_3CH + CO_2$$

The reaction was shown to be 90% complete in two hours at 100 degrees C. The kinetics of this reaction have been studied in some detail by Stewart [15] who found kinetic and isotopic evidence to support a carbonium ion rearrangement in which the hydride ion was transferred from the formate to the carbonium ion. Attempts to reduce the hydroxy function on other types of carbinols have given inconsistent results.[14] Simple reductions do not occur but rather rearrangements and the formation of double bonds takes place. Dihydroxy compounds have been shown to undergo dehydration to unsaturated compounds when refluxed with formic acid.

$$C_2H_5CH_2 - \underset{OH}{CH} - \underset{OH}{CH_2} \xrightarrow[\Delta]{HCO_2H} C_2H_5CH{=}\ CHCH_2OH + C_2H_5CHOHCH{=}\ CH_2$$

Those hydroxy compounds that contain an acetylenic linkage alpha to the hydroxy function undergo a rearrangement to the corresponding aldehyde or ketone.[16]

$$(CH_3)_2\overset{OH}{C}\text{-}C{\equiv}CH \xrightarrow{HCO_2H} (CH_3)_2\ C{=}\ CH\text{-}CHO$$

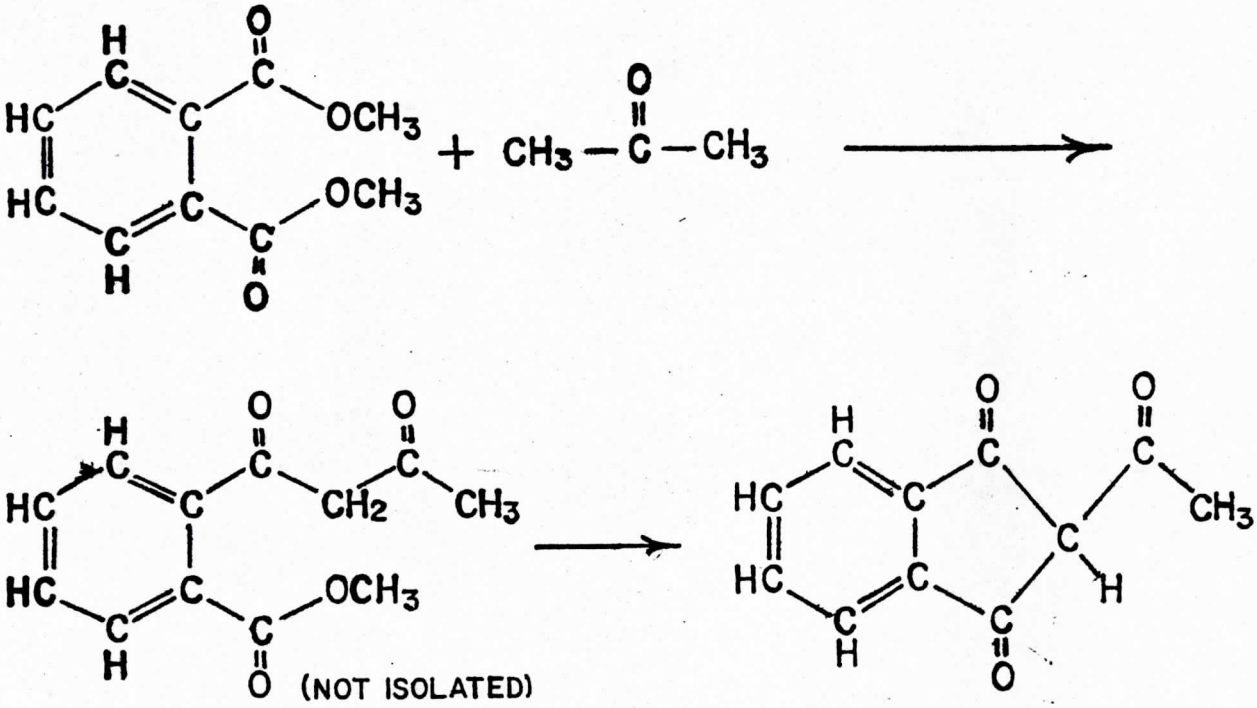
Formic acid esters of tertiary alcohols, when heated with formic acid and an acid catalyst, decompose into the

corresponding olefin. Most alcohols are converted into acids when treated with formic acid and concentrated sulfuric acid.[17] In the presence of most metal and acid catalysts, formic acid will decompose on heating into hydrogen and carbon dioxide. The molecular hydrogen is adsorbed on the metal surface and the system reduces oximes. These reductions, however, are quite similar to hydrogenations and cannot be considered true formic acid reductions since the formic acid only supplies the H_2.

DISCUSSION OF EXPERIMENTAL RESULTS

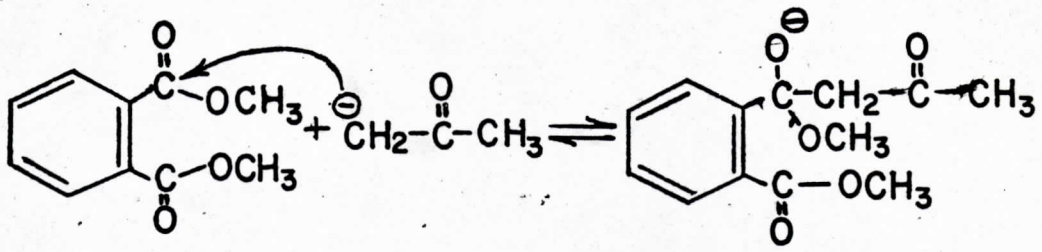
Preparation of 2-acetyl-1,3-indanediones

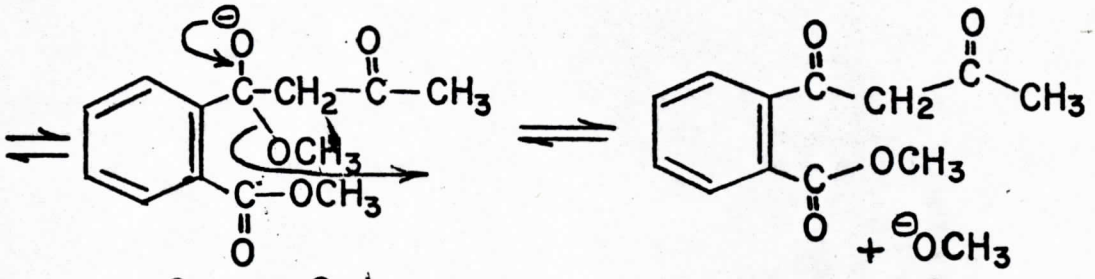
2-acetyl-1,3-indanedione was prepared by the intermolecular condensation and Dieckman type cyclization of dimethyl phthalate with acetone. This reaction may be catalyzed by strong bases such as sodium methoxide, sodium ethoxide, or sodamide [8]



The following mechanism, proposed by Hauser and Renfrow for the acetoacetic ester condensation, probably applies in this case, [19]

2.





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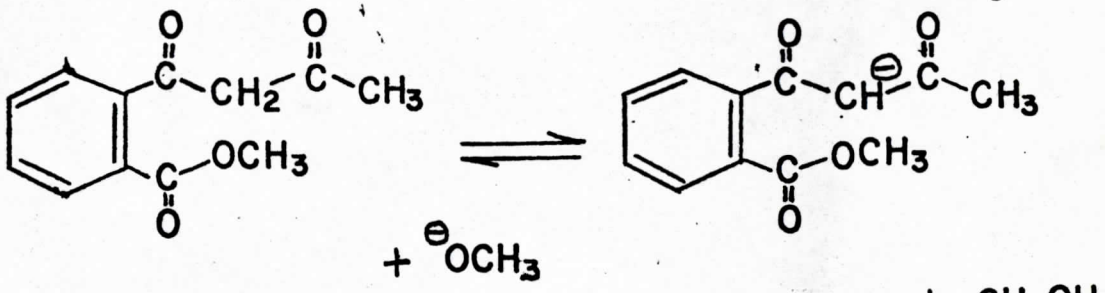
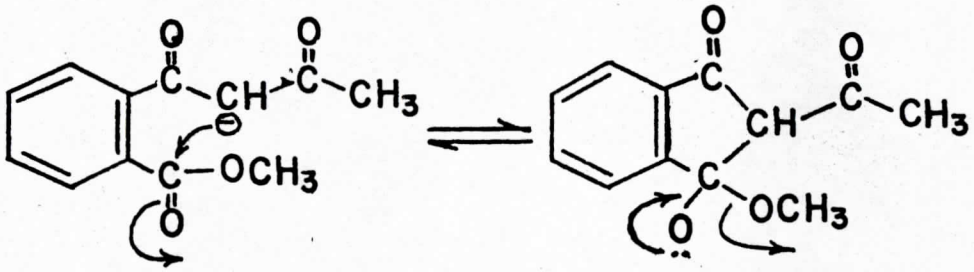
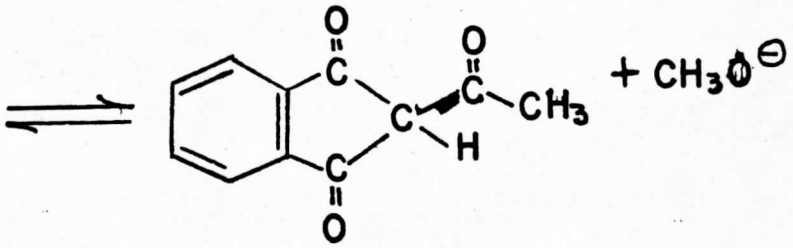




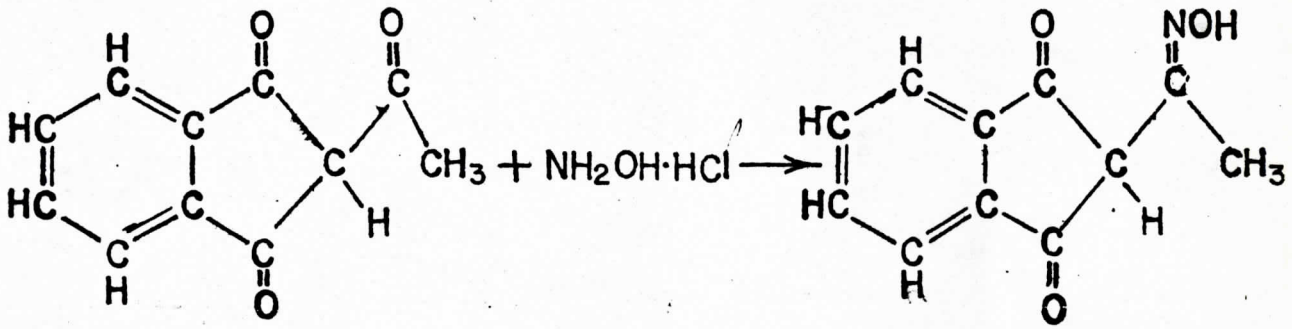


Conversion of Ketones to Oximes

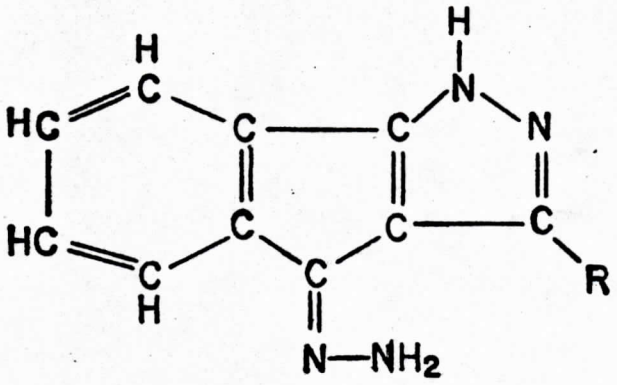
2-Acetyl-1,3-indanedione, along with nine other ketones, was converted into the corresponding oxime.

Four different types of reaction conditions were tried. No one set of conditions seemed to have a very extensive range of application. Details of each method are given in the experimental section and will not be discussed here.

The specific reaction under consideration in this work is as follows:



According to Mulliken,[20] a positive Tollen's test can be anticipated for oximes whenever the carbon atom of the carbonyl group that would be formed in case of hydrolysis is situated on an open chain, but it may not be satisfactory in cases where this atom is situated in a cyclic nucleus. Braun[21] was concerned with compounds of this type and in an attempt to determine whether a 1-hydrazone group on an indane ring would give a positive Tollen's test, the test was tried on compounds of the following type and in each case a negative test resulted.

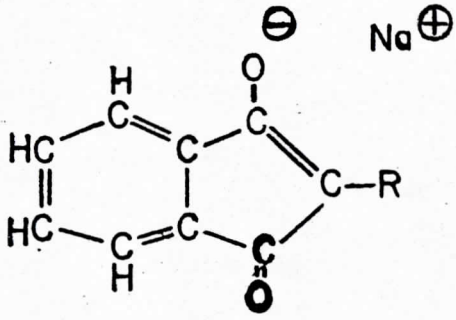


R = CH_3, C_2H_5

The monohydrazones of 2-acetyl, 2-propionyl, and 2-phenylacetyl-1,3-indanedione gave a positive Tollen's test while the monohydrazone of 2-diphenylacetyl-1,3-indanedione does not react. These tests indicated that in the monohydrazone of 2-diphenylacetyl-1,3-indanedione, the hydrazone function is situated on the side chain. Oxime formation is similar to the formation of hydrazones and should, therefore, follow similar rules. It was assumed that, in the case of 2-acetyl-1,3-indanedione, the oxime function is located on the carbonyl which is on the side chain. It is also assumed that there is no formation of oxime occurring on the other carbonyl functions which are situated on the indane ring.

There is other evidence that the monohydrazone, and the monoxime, forms on the side chain. Braun[22] reacted dilute aqueous sodium hydroxide with the monohydrazones of 2-acetyl, 2-propionyl, and 2-phenylacetyl-1,3-indanediones.

He found that each of these compounds dissolve readily and give a bright red solution. From the red solution can be recovered a red crystalline salt which also gives a positive Tollen's test. This indicates that the hydrazone group is still present and is not involved in the red color formation. The color of the red solution is the same as that given in aqueous base by 1,3-indanedione, 2-methyl-1,3-indanedione, or 2-phenyl-1,3-indanedione, which Hantzsch[23] proposed was due to the formation of a salt of the following type:



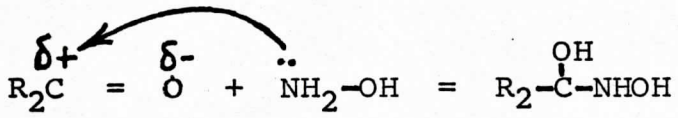
R= H, CH_3, C_6H_5

The red color of the alkaline solutions of the monohydrzzones of 2-acetyl-, 2-propionyl- and 2-phenylacetyl-1,3-indanedione strongly suggests the existence of the 1,3-indanedione group or its enol with the hydrazone on the side chain. 2-diphenyl acetyl-1,3-indanedione-1-hydrazone did not react with dilute aqueous base.

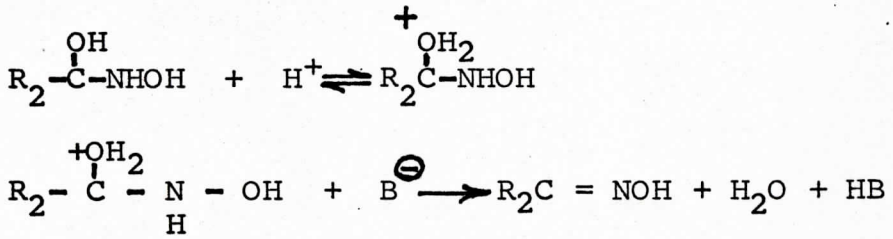
The infrared spectra of these materials confirm the proposed structures but suggest that these compounds are in the hydrogen bonded enol form, at least in the solid state. A brief consideration of spectra will be found in a later section.

Two mechanisms for oxime formation, one for acidic conditions and one for basic conditions, have been proposed.[24]

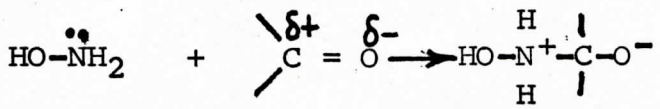
In acid media (general)

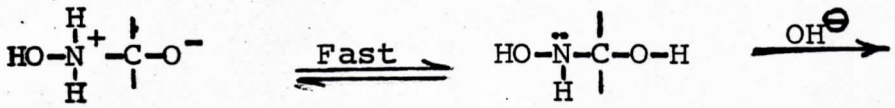


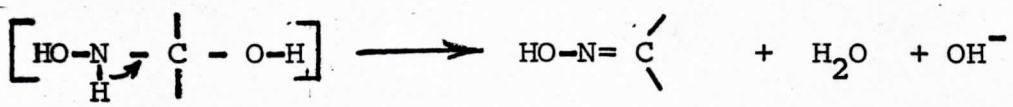
Essentially what occurs is that the electron pair on nitrogen acts as a nucleophile and attacks the partially positive carbonyl carbon.



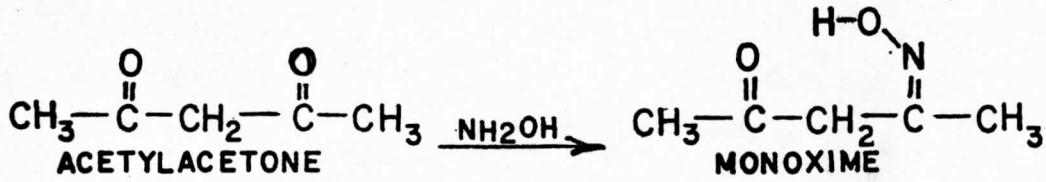
Base catalyzed mechanism (general)

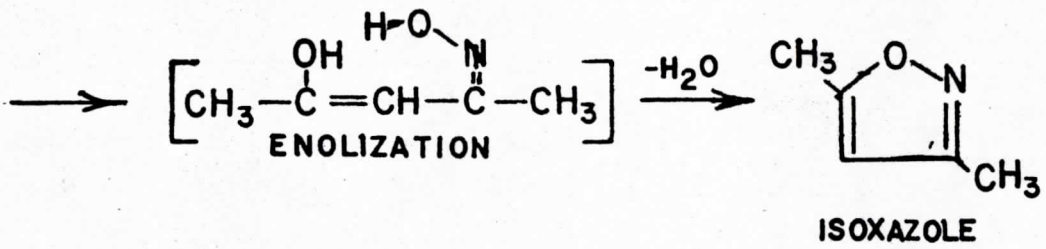






In β-keto structures, one secondary reaction which may be important is formation of the isoxazole ring structures.[25]



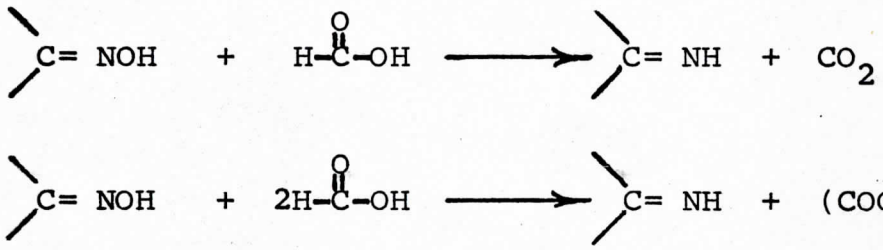


In this work, primary interest was given to the reduction of the oxime of 2-acetyl-1,3-indanedione. Several other oximes were prepared and subjected to the same reaction conditions to determine whether or not the reaction was general or specific, and were found to yield results similar to

those observed with 2-acetyl-1,3-indanedione oxime.

Reduction of 2-Acetyl-1,3-Indanedione Oxime

Formic acid, due to the presence of the aldehyde function, has been shown to be capable of reducing certain organic compounds, specifically certain trisubstituted carbinols for example. The major purpose of this project was to determine if formic acid could reduce the oxime function, and if so, what was the nature of the products.



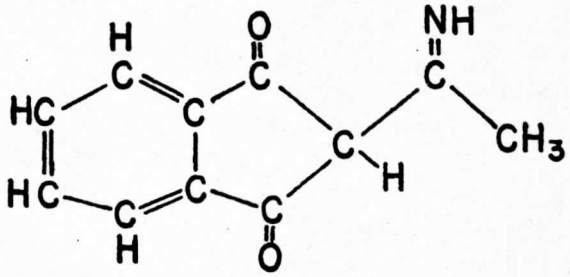
OR

The reaction was carried out by refluxing the oxime with formic acid for at least two hours. This was done under nitrogen to insure an inert atmosphere. It was initially assumed that the oxidized product would be carbon dioxide. After some effort was made to collect and measure this gas, it was decided that carbon dioxide did not form at all and that possibly a two electron reduction producing oxalic acid occurred instead. In this case, two

formic acid molecules would each supply one of the electrons required for the reduction resulting in the production of oxalic acid.

Reduction Product

Characterization of the reduction product was accomplished by adding the product mixture to water, making alkaline with sodium hydroxide and extracting with ether. After the ether was evaporated, the product was recrystallized from ethanol-water. The resulting compound had a melting point of 129-30 degrees C. The infrared spectrum was taken and supported the spectrum of a compound prepared by another method and shown by elemental analysis to have the molecular formula $C_{11}H_9NO_2$.[26] The only reasonable structure compatible with the accumulated data and reaction conditions is



(see spectrum 3)

2 acetimido-1,3-indanedione

There is some evidence that there is a significant amount of another material, other than oxime and imine, in the product mixture. This evidence is based on observations from thin-layer chromatography and also from the low yield of the reaction (13.1%). When the reaction mixture was chromatographed, there was always a separation of the spot into three regions. The original brown spot separated into a yellow region, a reddish region, and a trailing yellow-brown region. When the column was used, there was again a yellow band which was well defined and this was followed by a darker band. The red band may have been present but, due to the color of the column itself, could not be seen.

There are several possible reactions which could account for the presence of the third component in the product mixture. Of these, the best choice is a disproportionation reaction between molecules of the starting material. This would account for the formation of imine since the disproportionation would involve an oxidation-reduction couple. This possibility was not investigated in any detail and is not considered further.

Oxidation Products

When 2-acetyl-1,3-indanedione oxime was refluxed with formic acid, there was very definite evidence that a reaction did occur. There was a color change from a clear yellow solution to a heavy dark brown colored solution. This color change was usually complete after about 20 minutes of heating.

Since there was good evidence that 2-acetyl-1,3-indanedione oxime was reduced to the corresponding imine in hot formic acid, the next step in complete characterization of the reaction was to identify the possible oxidized product. The first assumption was carbon dioxide and several techniques were used to detect this gas.

Carbon dioxide combines with calcium hydroxide in solution (limewater) to form an insoluble precipitate of calcium carbonate. A portion of 2-acetyl-1,3-indanedione oxime was refluxed with formic acid in a system in which any gas that formed was passed through a gas dispersion disc into a saturated calcium hydroxide solution. After heating for 2-3 hours, no evidence of any calcium carbonate formation was observed. The calcium hydroxide solution was replaced by barium hydroxide (sat.) and the situation reproduced with

the same results; no barium carbonate formation was observed.

Another method that was tried was collecting any gas evolved in gas collecting bottles by the downward displacement of water. The reaction vessel was connected to a delivery tube which in turn opened into a gas collecting bottle. First, distilled water was used as a control; secondly, an equal amount of formic acid; and finally, a sample of the oxime and formic acid were all heated to reflux for a given time. There was again no evidence of any carbon dioxide being generated by the reduction.

Another technique that was used in an effort to detect any carbon dioxide was absorption by Ascarite (NaOH on asbestos). Any gas leaving the reaction vessel was passed in turn through a calcium chloride drying tube, a saturated solution of sodium benzoate to remove any formic acid vapor, and a drying tube packed with Ascarite. Ascarite quantitatively absorbs carbon dioxide. The tube was carefully weighed before and after three hours of heating. Again, there was no evidence of carbon dioxide.

At this point, it was decided to abandon the search for carbon dioxide since there was absolutely no evidence that it was a product. Another possibility for the oxidized

product was oxalic acid. Detection of oxalic acid, in the presence of formic acid, proved to be an interesting problem and several techniques were used to solve it.

Thin-layer chromatography was tried as a method for separating oxalic acid from the other components in the product mixture. No visualization reagent could be found which would discriminate between oxalic acid and formic acid, so it became necessary to separate these two acids from each other also. Oxalic acid is very tenacious and the largest R_f value found in the literature was 0.17.[27] This was on polyamide using a mixed solvent system of acetonitrile-ethylacetate-formic acid (82: 9.1: 9.1). This value was very nearly reproduced (0.13) in this work, on control strips. However, when a sample of the reaction mixture was chromatographed, detection proved more difficult. Therefore, due to its low R_f value, better results were obtained by separating the other components from the oxalic acid. To do this, several solvent systems and three different absorbents were investigated. Very little success was obtained using alumina plates. However, both silica gel and polyamide proved to be of some value. A number of different solvent combinations were experimented with as a means of developing the chromatograms. Some success was achieved

by developing the chromatograms in two stages. Since the indane components all have a dark color, they tend to mask the colorless acids. The indane components were separated first and then a second solvent system was used to separate the acids. Several systems were found that would do this quite well. n-hexane-chloroform, n-butanol-benzene, benzene-cyclohexanone, and n-butanol-chloroform all moved and separated the indane components easily. Separation of the acids was more difficult. Oxalic acid exhibited a measurable R_f value only on polyamide and this was not precisely what was reported in the literature.

In each system three strips were developed simultaneously. One spotted with oxalic acid, one with formic acid, and finally the product mixture. As the solvent front ascended, the product mixture spot moved along with it. Separation of the visible components of the reaction mixture could first be noticed about one-third of the way up in the strip. By the time the solvent front reached the top of the strip, the original brown spot had separated into three regions; a yellow region nearest the top, a red region below this, and a trailing brown-yellow region. When these chromatograms were visualized, there was very little migration of the acids. If a second phase development was to be

attempted, the strips were always dried at 90°C. to remove the solvents.

Although some success was attained, using the controls, with this technique, no definite proof of the presence of oxalic acid in the product mixture was obtained. The R_f value could not be correlated with the value observed on the oxalic acid control strip. Generally, it was concluded that, first, if there is any oxalic acid generated, it is not enough to account for the amount of product recovered; secondly, there is a significant amount of a third component in the product mixture.

Column chromatography was also used and supplied some information about the nature of the product mixture. The most difficult problem encountered with this technique was detection of the various components in the column, and specifically the acids. According to Marvel and Rands [28] and Kesner and Muntwyler, [29] oxalic acid can be separated from formic acid on a hydrated silica gel column. No visual indicator was found that could be used to titrate aliquots of the eluant with a base. It was thought that perhaps a potentiometric titration could be used. The problem encountered here was that the pH meter would not stabilize in the nonaqueous media. No reproducible results were obtained using this technique, especially at low acid concentrations. Some

of the results of these efforts were reported by plotting mV or pH versus percentage composition of solvent. The results were discouraging since no linear relationship was found. Also, meter response versus formic acid concentration in various solvent systems was investigated. Again, no strict relationship was found and this technique was abandoned.

The one system that was found to be useful was a column using a combination of diatomaceous earth (Celite 545) and sucrose as the stationary phase and impregnating this with alphamine red-R, an acid-base indicator.[30,31] To eliminate any complications due to the presence of water in the system, a small amount of barium sulfate was included at the top of the column.

As controls, oxalic acid, formic acid, and oxalic acid dissolved in formic acid were chromatographed. Formic acid passed through the column readily, but oxalic acid proved to be quite tenacious and moved only slightly. A good separation of the mixture was observed.

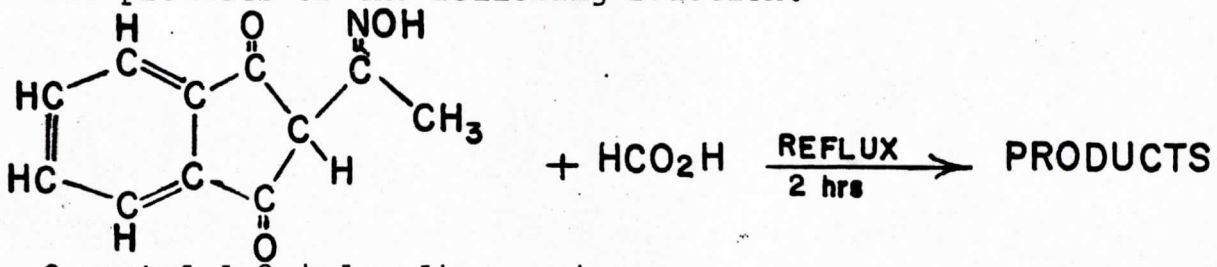
A sample of the product mixture was applied to the column. A blue band, implying an organic acid, appeared quickly, moved down the column as a well defined front, and then the original red color of the column reappeared. There was also

observed a yellow band followed by a darker brown-yellow band. These are believed to be the indane components of the product mixture.

As with the thin-layer systems studied, there was no conclusive proof of any significant amount of oxalic acid in the products. It first appeared as if there was a small amount of oxalic acid at the top of the column, but this residual blue color appeared even if 97-100% formic acid was applied to the column. The same conclusion was formed from the evidence of this technique, that is, very little, if any, oxalic acid was present. Also, there were other colored bands observed in the column as there were in the thin-layer chromatograms. The order of appearance was similar with a yellow band leading, followed by a darker brown-yellow band. The central red band observed on the thin-layer sheets could not be seen in the column.

Conclusions

The original objective of this project was to determine the products of the following reaction:



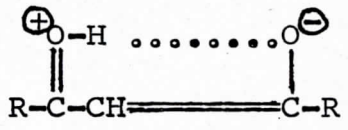
2-acetyl-1,3-indanedione oxime

The oxime has been shown to be reduced to the corresponding imine. The two most logical choices as to the identity of the oxidation products were carbon dioxide and oxalic acid. However, no evidence was found to support the existence of either of these compounds. The reaction appears to be somewhat more complicated than was originally suspected. An alternative which seems to have the most merit is that a disproportionation reaction occurs. In this case, molecules of starting material react with each other in an oxidation-reduction type system. There are other possibilities but this best accounts for the reduction product that is formed and could also explain the existence of the third component found in the product mixture.

INTERPRETATION OF SPECTRA

Infrared Spectra of 1,3-Indanediones

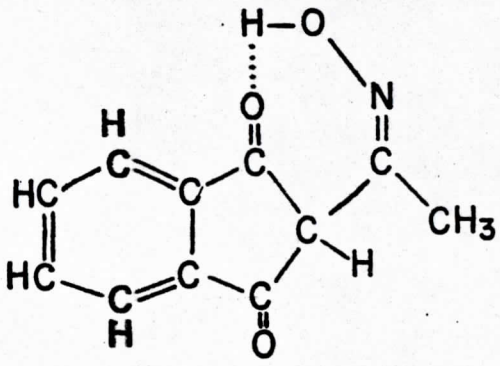
Braun reports a systematic study of the spectra of 1,3-indanediones, although he did not report on the 2-acetyl compound. Until this time, there had been no comprehensive study of compounds of this type. A considerable amount of work has been done on the simpler β-diketones.[32,33] According to Rasmussen, et.al., [34] acetylacetone and other related compounds exist largely in the mono-enol form. These compounds fail to show any absorption in the normal conjugated ketone region. These compounds do, however, show very strong absorption in the 1640-1540 cm^{-1} range. This absorption is in the form of an intense, broad band. This is probably a result of the fact that the double bond character of the carbonyl group is reduced by resonance.[34] The term "conjugate chelation" has been applied to this effect.



Infrared Spectrum of 2-Acetyl-1,3-Indanedione Oxime

Oximes characteristically absorb at 1640 cm^{-1}, due to the carbon-nitrogen double bond. Also, according to Palm and Werbin,[35] certain β-ketoximes can be distinguished by the position of the oxygen-hydrogen stretching frequency around 3190 cm^{-1}. The spectrum of 2-acetyl-1,3-indanedione oxime showed a small, sharp peak at 3195 cm^{-1} (see spectrum 2).

As far as is known, no systematic study has been done on the spectra of compounds similar to 2-acetyl-1,3-indanedione oxime. Resonance structures similar to those drawn for the corresponding ketone could be an important contribution to the actual structure.



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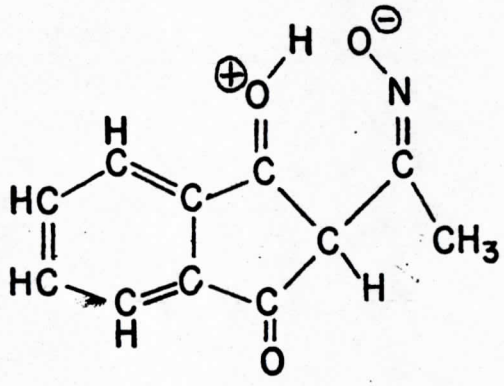


II

The fact that hydrogen bonding is possible, as in structure I, would also influence the orientation of atoms in the molecule. This would affect the spectra as well as the properties

and reactions of this compound. Due to the nature of the instrument used to record the various spectra, it was not possible to detect any shift to a longer wavelength because of bond delocalization. If there was any shift, it would probably be small since the anti- form of the oxime would be stearically favored over the syn-. Only in the syn- isomer would intramolecular hydrogen bonding be possible.

Infrared Spectra of 2-Acetyl-1,3-Indanedione Imine

Ketimines characteristically absorb in the same region as the corresponding ketone and oxime, 1610-1640 cm^{-1}. Again, as far as is known, no organized study has been done on the infrared spectra of this and other related compounds. (See spectrum 3).

Suggestions for Further Investigations

An organized and comprehensive study of the various spectra of 2-acetyl-1,3-indanedione oxime and other related compounds along with the corresponding imines needs to be done. This study should provide valuable information about the structure of these compounds. An effort should also be made to isolate and identify the other indane products.[9] The possibility that secondary reactions are occurring should

be considered in more detail, especially a disproportionation reaction.

EXPERIMENTAL

Melting Points

All melting points are in degrees Celsius and are uncorrected. A Fisher-Johns hot plate-type apparatus was used in each case.

Spectral Analyses

Spectra of the various compounds were obtained using a Perkin-Elmer Model 137 recording spectrophotometer. In all cases, the spectra were taken using the nujol mull technique.

Preparation of 2-Acetyl-1,3-Indanedione

The 2-substituted indanedione was prepared following the method of Braun.[36] To prepare the sodium methoxide, sodium metal (0.667 mole or 15.3 g) was heated in a three-necked round bottomed flask in 250 ml. of toluene with vigorous stirring. After the sodium melted, vigorous stirring was continues as the mixture was cooled to room temperature. The toluene was siphoned off with a pipette and 300 ml. of dry benzene added. The benzene had been dried by twice shaking it with anhydrous calcium chloride and filtering.[37] With stirring, dry methanol (22.0 g, 0.69 mole, twice distilled) was added slowly and the mixture was refluxed.

Braun's method was altered slightly at this point. It was found to be necessary to add more methanol to completely convert the sodium into sodium methoxide. Methanol addition was continued until no sodium metal remained. The condenser was then replaced with a still head and the excess methanol was removed by distillation. The distillate was checked periodically with ceric nitrate solution to test for methanol.[38]

To prepare the ketone, a mixture of dimethyl phthalate (129.2 g, 0.667 mole) and acetone (0.667 mole, dried by distillation) was added to the same reaction vessel over a period of about thirty minutes. Distillation was continued to remove any methanol produced during the condensation. To test for methanol, a few drops of the distillate were tested with ceric nitrate reagent. The time required for distillation may be as much as ten hours or more. Upon cooling, a yellow-brown solid forms which is filtered and dried. The solid is then suspended in 500 ml. of water and made acid to litmus with concentrated HCl. The yellow indanedione is filtered, dried and recrystallized from ethanol-water. The final crystals were dried at 80°C. with a yield of 57 g. after recrystallization (45.6%). The melting point observed was 108.5-109.5°C. compared to an accepted value of 108°C.[39]

Conversion of Ketones to Oximes

Pyridine Method[40]

A mixture of about 1.0 g of the aldehyde or ketone and 1.0 g of $NH_2OH \cdot HCl$ is added to a solution of pyridine and absolute ethanol (5 ml. each), in a 100 ml. round-bottomed flask with ground glass joints. The mixture is refluxed under the hood for two hours. The solvents are removed by evaporation under a hood. The residue is dispersed completely in 5 ml. of cold water and then filtered. The oxime is then recrystallized, usually from ethanol-water.

An attempt was made to prepare the oxime of each of the following compounds using the pyridine method:

Compound	Results	Melting Point	
		Observed	Reported
cyclohexanone	good	90-91°	90°
benzophenone	good	143-44°	141°
vanillin	good	118-19°	117°
benzoin	oil	-	-
acetylacetone	no product	-	-
anthraquinone	no product	-	-
benzil	no product	-	-

No attempt was made to measure percent yield by this, or any other, method. A yield is described as good if a substantial amound of the product was formed and had a sharp melting point, within 1-2 degrees of that listed in the literature.

Potassium Hydroxide in Ethanol[41]

Two grams of the ketone were mixed with 2.0 g of $NH_2OH \cdot HCl$. To this was added 8.0 g of KOH and 40 ml. of 95% ethanol. The mixture was heated under reflux for two hours and fifteen minutes and poured into 300 ml. of water. The mixture was allowed to stand to allow any unreacted ketone to separate. The mixture was then filtered, acidified with HCl and allowed to stand so that the oxime could crystallize. The product is recrystallized from ethanol.

An attempt was made to prepare the oxime of each of the following compounds using the potassium hydroxide method:

Compound	Results	M. P. Observed	M. P. Reported
benzoin	poor	210°	151°
camphor	good	117-18°	118°
acetylacetone	poor	-	149°

Sodium Acetate Method[26]

Add 0.25 g of the ketone to 10 ml. of 95% ethanol. Add to this 1.0 g of sodium acetate 1.0 g of $NH_2OH \cdot HCl$. If the crystals do not dissolve, heat the mixture gently until the alcohol boils. If solution still does not occur, add H_2O drop by drop until it does. Reflux this for ½-1 hour. Add water drop by drop until the first indication of a cloudy precipitate occurs. The oxime should precipitate.

An attempt was made to prepare the oxime of each of the following compounds using the sodium acetate method:

Compound	Results	M. P. Observed	M. P. Reported
2-acetyl-1,3-indanedione	good	210-13°	-
acetylacetone	poor	-	149°

Sodium Hydroxide Method[41]

One gram of $NH_2OH \cdot HCl$ was dissolved in 6.0 ml. of water; 4 ml. of 10% NaOH and 0.4 g of the ketone was added. Just enough ethanol was added to allow solution to occur. The solution was warmed for 10 minutes and placed in an ice bath.

An attempt was made to prepare the oxime of each of the following compounds using this method:

Compound	Results	M. P. Observed	M. P. Reported
acetylacetone	good	151°	149°
benzoin	poor	-	151°

It was suspected that the pH and the alcohol-water composition were important in oxime formation, but this was not investigated further. A solvent boiling point that is too high, i.e., above the melting point of the oxime or ketone, may result in the formation of an oil and inhibit the formation of crystals. The oxime of acetylacetone was expecially difficult to prepare. This difficulty could possibly be due to the formation of an isoxazole structure. Characterization of the various oximes in the above tables was by comparison of melting points and by use of the $FeCl_3$ test.[42]

Reduction of Oximes

The various oximes were refluxed with formic acid for two hours. This was done by taking 1.0 g of the material and adding 25 ml. of 97-100% formic acid (Fisher Chemicals) which had been previously distilled. The reduction was carried out in a 100 ml. round-bottomed flask with 24/40 ground glass fittings. Provision was made for removal of

air by using a nitrogen atmosphere. Nitrogen was first bubbled through formic acid in an adjacent test tube with a side arm, then the gas passed over the mixture in the reaction vessel. The nitrogen was passed through the system for about 5 minutes before heating was begun. Nitrogen was flushed through the system during the entire heating period. A condenser was fitted to the reaction vessel and provision was made for measuring any gas evolution. A connecting adapter was fitted into the top of the condenser and a cold finger fitted to the top of the adapter. All connections were not lubricated in an effort to avoid contamination.

Determination of Products

The Reduction Product - After two hours of heating, the reaction mixture was allowed to cool and then poured into water. The water mixture was then made alkaline with concentrated NaOH and extracted with diethyl ether. The ether was then evaporated by an air stream under a hood and the imine was recrystallized from an ethanol-water mixture. A yellow crystalline material was obtained upon recrystallization which melted at 231-33^{o}C. Analysis of the reduction product was done by Alfred Bernhardt in Mulhein, Ruhr West Germany. The analysis number in 10319, and the results

were as follows: %C = 70.48, %H = 4.86, %N = 7.36, and %O = 17.30 (by difference). From this information, the empirical formula was calculated and found to be $C_{11}H_9O_2N$. The experimental percentages were very close to the calculated values for this compound, %C = 70.59, %H = 4.81, %N = 7.48, %O = 17.12. 0.120 g was obtained from 1.0 g of the oxime after recrystallization for a percent yield of 13.1%. The infrared spectrum and melting point were recorded. Both the spectra and melting point matched that of a compound prepared previously in a different, but related, work.[43] This product was then assumed to be the imine.

The Oxidation Product - The determination of carbon dioxide was attempted by several methods. 0.200 g of cyclohexanone oxime was added to 20 ml. of formic acid. The system was flushed with nitrogen and brought to reflux temperature. After heating for two minutes, gas evolution began, voluminously at first, but somewhat sporatically. Gas evolution completely ceased after 5 minutes and was assumed to be due to the thermal expasniom of the gas in the reaction vessel. Heating was continued for 75minutes with no further evolution of a gas. The materials in the reaction vessel turned a light brown color after prolonged heating.

Similarily, the oximes of 2-acetyl-1,3-indanedione, camphor, benzophenone, acetylacetone, and vanillin were refluxed in the same manner as described above with similar results. The evolution of a gas from the system was noticed early in the heating cycle which ceased, in each case, in 5-7 minutes. In none of these cases was there any evidence of $CaCO_3$ formation in the $Ca(OH)_2$ vessels, and the gas evolution was again believed to be due to the expansion of air in the reaction vessel.

As a control, 25 ml. of formic acid was heated at its boiling temperature, under nitrogen, for two hours in an attempt to determine if it decomposed significantly under these conditions. Two gas collecting bottles were filled approximately two-thirds full with saturated $Ca(OH)_2$ solution (as above) and connected to the system through the second adapter. About 12 minutes after heating was begun, a gas began passing through the $Ca(OH)_2$. The evolution ceased after about ten minutes of heating. Heating was continued for two hours and no more gas evolution was observed. Again, there was no evidence of any $CaCO_3$ formation in either of the $Ca(OH)_2$ vessels, and the gas evolved during the early heating period was attributed to expansion of air.

Also as a control, 25 ml. of formic acid was heated as above; but in this instance, two drops of concentrated sulfuric acid was added to the system. Gas evolution began immediately and continued through the entire heating peridd, ca. one hour. Again, there was no evidence of any $CaCO_3$ formation.

0.5 g of oximes of camphor, benzophenone, acetylacetone, and vanillin was heated with 20 ml. of formic acid for 75 minutes. In every case, the evolution of gas was observed early but ceased after five to ten minutes. 25 ml. of distilled water was placed in the round-bottomed flask and the system was flushed with nitrogen as before. The water was heated and the same effect was observed.

A similar procedure was followed in which the saturated $Ca(OH)_2$ solution was replaced with saturated $Ba(OH)_2$. The conditions were simulated to those already described and the results were the same. There was no evidence of any $BaCO_3$ formation in any of the cases.

25 ml. of distilled water was heated in a round-bottomed flask and provision was made for collecting any gas evolved in a flask by the downward displacement of water. After the evolution of gas from the system had ceased, the volume of

gas collected was measured by readding water and determining how much was required to refill the flask. The same procedure was followed with 90% formic acid (Fisher Chemical Co.) and 1.0 g of 2-acetyl-1,3-indanedione oxime added to 25 ml. of formic acid, boiling point of 100°C. The results were as follows:

System	Volume of Gas Collected
25 ml. distilled water	99.40 ml.
25 ml. formic acid	112.90 ml.
oxime + 25 ml. formic acid	110.30 ml.

In each case, the time of heating was carefully monitored.

Another attempt was made to detect carbon dioxide by heating 25 ml. of formic acid at reflux for 3 hours and passing the volatile components through the following system:

(1) A calcium chloride drying tube

(2) 2 gas scrubbing bottles fitted with fritted disc exits for gas dispersion and filled with saturated sodium benzoate. The purpose for including these was to remove any HCOOH vapor that passed the condenser. The formic acid vapors would be converted to sodium formate and remain in the solution:

$$\text{Ø-COO}^{\ominus} + \text{HCOOH} \longrightarrow \text{HCOO}^{\ominus} + \text{ØCOOH}$$

The equilibrium for this reaction favors the proton being attached to the ϕ-COO^- rather than the $^-O-\overset{O}{\overset{\|}{C}}-H$.

(3) An Ascarite tube to absorb carbon dioxide quantitatively.

$$2NaOH + CO_2 \longrightarrow \underline{Na_2CO_3} + H_2O$$

The results were as follows:

Sample	Ascarite Tube Orig.	Final	Wt. Gain
1. 25 ml. formic acid	54.1651 g.	54.1969 g.	0.0318 g.
2. 1.0 g. of 2-acetyl-1,3-indanedione oxime in 25 ml. formic acid	53.6266 g.	53.6498 g.	0.0232 g.

The test was repeated with similar results (Ascarite tube showed only 0.0210 g. weight gain). The formic acid was distilled once before this test was tried. Ca. 75 ml. was placed in a 250 ml. distilling flask with 24/40 ground glass joints. The first 5-10 ml. were discarded and the last 15-20 ml. not collected. The Ascarite used was from Arthur H. Thomas Co., Philadelphia, Pa., U. S. A. (8-20 mesh, lot #7423).

Determination of Oxalic Acid

Nessler's Solution[44]- Two drops of the reaction solution were taken from the reaction vessel after two hours of refluxing and added to a microcrucible. The product was taken to dryness by adding several drops of concentrated ammonia followed by heating at 115°C. for several minutes. After cooling, a drop of Nessler's solution was added. A red-brown precipitate, characteristic of oxalic acid, was observed. However, the same test was applied to twice distilled formic acid and gave the same results.

Partition Chromatography [28,29,30,31]- Some information and data was accumulated while trying to find an applicable solvent system for use with potentiometric titrations to detect the acid content of the column eluant. Also, standard acid solutions were prepared in an effort to determine the sensitivity of the pH meter to low acid concentrations in nonaqueous media. The meter was read in pH units and adjusted to read 14 on standby in pure solvent. The graphs of this data were mentioned earlier and proved to be of little value. The most obvious result was that the meter did not stabilize in the nonaqueous media considered. An effort was made to reproduce conditions for each reading.

Also, the meter did not respond linearly to changes in the acid concentration.

Stationary Phase - 75 g. of Celite (diatomaceous earth or kieselguhr) was placed in a Waring Blender and 750 ml. of a mixture of n-hexane and acetone (1 to 1) added. The finer grade of Celite was also tried and found to be less satisfactory because the texture was too fine and a reasonable flow rate in the column could not be maintained. To this was added 30 ml. of a sucrose-water mixture (2 parts sucrose - 1 part water by weight), twelve milliliters of alphamine red-R indicator solution, and 0.5 ml. of 0.1N H_2SO_4. The mixture is blended at a very low speed until the proper consistency is obtained. Best results are obtained if the packing material remains coarse. If blending is prolonged, or is too fast, the slurry becomes too fine and the developing phase is retained, or moves only slowly. The resulting material should have the texture of a coarse sand with a red-orange color. A small amount of $BaSO_4$: Celite (3 to 2) cap material is added as a slurry with n-hexane-acetone and loosely packed to remove any water which might be present. The stationary phase is stored in a glass-stoppered bottle at 4°C. and may be kept for several months.

Developing Phase - The solvents used in the various combinations were not distilled, dried, or otherwise purified. All solvents were filtered after mixing and before they were added to the column. The best results for this particular system were obtained using chloroform and n-butanol (75:25) although several others were tried and found to be satisfactory.

Columns - The columns used had an inside diameter of 1.5 cm and were packed to a height of ca. 10 cm. An effort was made to maintain the flow rate at about 2 ml./min. Care must be taken to avoid air pockets in the column. A small plug of nonabsorbent glass wool was used to contain the absorbent. A description was given in the discussion section.

Addition of Samples - 1.0 ml. (usually) of the sample was added from the top by using a graduated pipette or a dropper. The column capacity may be as much as 2 ml. or more, depending on column height and degree of packing.

Good separations were observed in the column. This technique shows much promise in separations of this type. Several extensions, such as acid-base titrations and qualitative analysis of organic acids, are possible. One advantage of this technique is the ability to visually monitor the

progress of the various components in the column.

Thin-Layer Chromatography[27,45,46,47,48] -

Stationary Phase - Pre-coated silica gel sheets on aluminum (without fluorescent indicator). 20 x 20 cm with a layer thickness of 0.25 mm. Prepared by E. Merck AG. Darmstadt, Germany and distributed by EM Reagents Div., Brinkmann Instruments, Inc., Westbury, NY, 11590. Precoated polyamide TLC sheets (polyamide 11-F-254) on aluminum. 20 x 20 cm with a layer thickness of 0.15 mm. Also from E. Merck.

Developing Phase - The solvents were distilled before use in some cases but it was found that satisfactory results could be obtained using the reagents directly from the bottle. A wide variety of solvent systems were tried. This technique was studied as a means of separation and not intended to be quantitative. No effort was made to obtain R_f values of the various components. In each case, the reaction mixture being studied was run simultaneously with formic acid and oxalic acid controls. Identification was by direct comparison. The results are described as good, fair, or poor. For a system to be described as good, there must be a very definite separation into bands and each band must be independent of the other bands. There were a few cases where

a well defined separation did not occur, but there was some evidence that the solvent composition could be altered slightly to give better results. These situations are classified as fair. Poor results gave no indication of a separation. The following is a list of most of the solvent combination systems that were tried.

Stationary Phase	Developing Phase	Results
silica gel	C_6H_6-CH_3OH-CH_3COOH (45:8:4)	poor
	C_2H_5OH(95%)-H_2O-NH_4OH (100:12:16)	poor
	Chloroform-n-BuOH (50:50)	good
	C_6H_6-Dioxane-CH_3COOH (90:25:4)	poor
	n-BuOH	fair
	n-BuOH-Dioxane-CH_3OH (100:25:25)	poor
	$CHCl_3$-n-BuOH-CH_3OH-Dioxane (1:1:1:1)	poor
	Chloroform-n-BuOH (75:25)	good
	C_2H_5OH	poor

Stationary Phase	Developing Phase	Results
silica gel	isopropylalcohol-ethyl-acetate-H_2O (24:65:12)	poor
	CH_3CN-$C_2H_5CO_2CH_3$-HCO_2H (82:9:9)	good
	HCO_2H-$C_2H_5CO_2CH_3$-H_2O (1:3:1)	good
	EtOH(95%)-H_2O-NH_4OH(25%) (80:7 • 5:12 •5)	poor
	C_6H_6-CH_3OH-CH_3COOH (79:14:7)	poor
	$C_4H_9CO_2CH_3$-C_4H_9OH-HCl (2:1:0.3)	poor
	C_5H_5N-$C_2H_5CO_2CH_3$-MeOH (1:2:0.8)	fair
	C_4H_9OH-HCO_2H-H_2O (15:10:15)	good
	a-C_4H_9OH-$CHCl_3$-C_5H_5N (25:25:10)	poor
	b-C_6H_6	
polyamide	CH_3CN-$C_2H_5CO_2CH_3$-HCO_2H (82:9:9)	good

Stationary Phase	Developing Phase	Results
polyamide	isopropyl alcohol-$C_2H_5CO_2CH_3$-H_2O	
	(24:65:12)	poor
	HCO_2H-$C_2H_5CO_2CH_3$-H_2O	
	(1:3:1)	poor
	$C_4H_9CO_2CH_3$-C_4H_9OH-HCl	
	(2:1:0.3)	poor
	C_5H_5N-$C_2H_5CO_2CH_3$-CH_3OH	
	(1:2:0.8)	poor
	a-C_4H_9OH-$CHCl_3$	
	(50:50)	good
	b-CH_3CN-$C_2H_5CO_2CH_3$-HCO_2H	
	(82:9:9)	
	a- n-Hexane-$CHCl_3$	
	(50:50)	good
	b-CH_3CN-$C_2H_5CO_2CH_3$-HCO_2H	
	(82:9:9)	

Visualization -[45,46,47]

Sulfuric Acid Reagents - Reagent A-equal volumes of concentrated H_2SO_4 and methanol are cautiously mixed (cooling). Reagent B-5% solution of concentrated H_2SO_4 in ethanol. The

chromatogram is sprayed with the reagent, allowed to dry for 15 minutes in the air and then heated at 115°C. until the color has reached a maximum. Most organic compounds are charred leaving black spots.

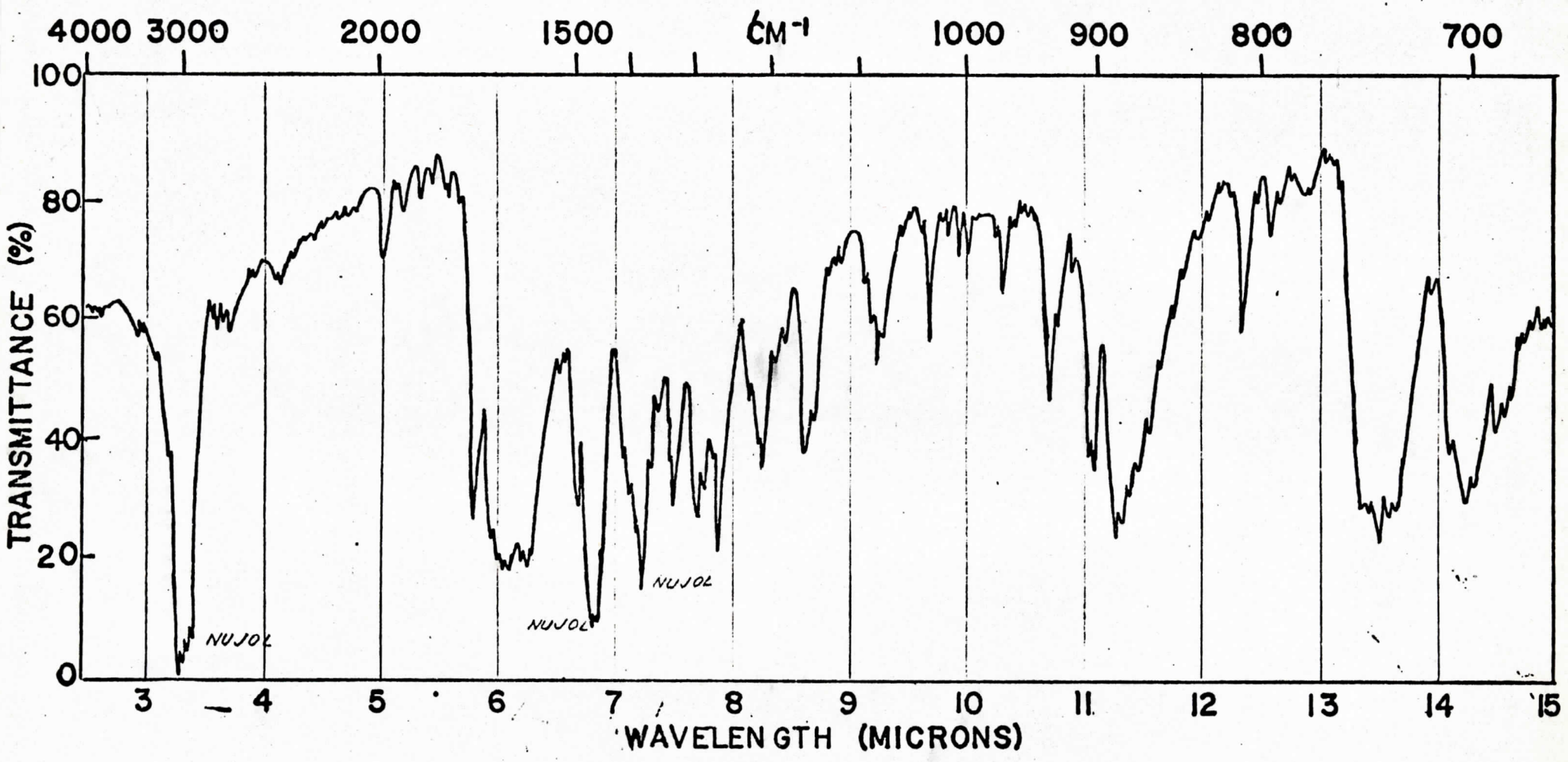
Bromocresol Green - Bromophenol Blue - Potassium Permanganate Reagent - Solution a- 0.075 g. of bromocresol green and 0.025 g. bromophenol blue are dissolved in 100 ml. absolute ethanol. Solution b- 0.25 g. of potassium permanganate and 0.5 g. of sodium carbonate ($Na_2CO_3 \cdot 10H_2O$) are dissolved in water and the volume made up to 100 ml. Spray reagent - solution a and b are mixed 9:1 (volume) and used immediately for spraying; the mixture can be kept only 5-10 minutes. Yields yellow spots on a blue background.

Bromocresol Purple Reagent - 0.04 g. of bromocresol purple is dissolved in 100 ml. of 50% ethanol and the pH adjusted to 10.0 (glass electrode) using 0.1N NaOH. The TLC layers are heated for 10 minutes at 100°C. After cooling, they are sprayed and yield yellow spots on a blue background.

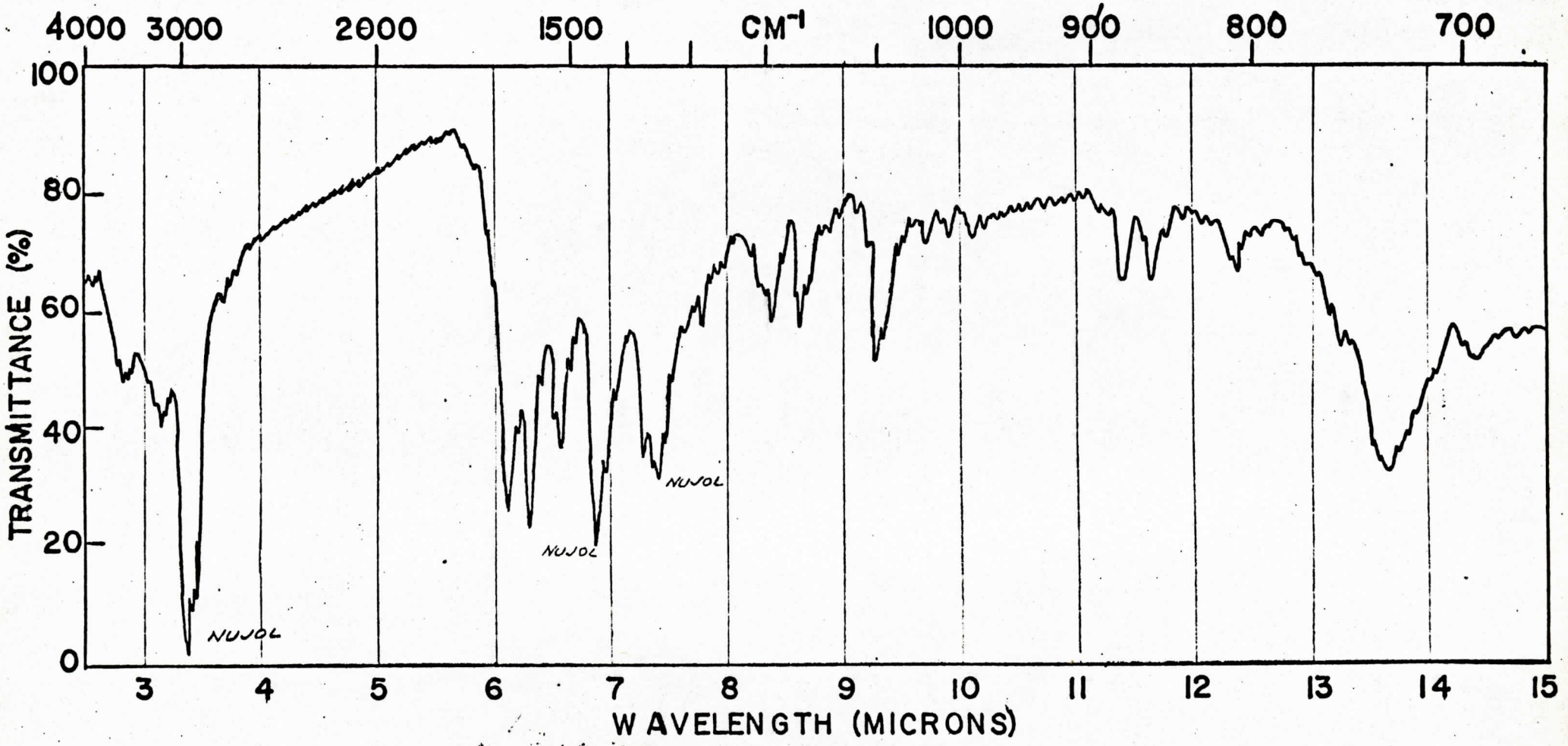
All the spray reagents were applied with a two-part glass sprayer with ground glass joints for atomizing reagents with compressed air (Fisher Scientific Co.).

The plates were spotted with drawn out melting point capillary tubes. The 20 x 20 cm. plates were usually cut into strips ca. 3 x 20 cm. The strips were spotted ca. 3 cm. from the bottom, above the solvent level. The chamber was an Eastman Chromatogram Chamber Plate Set from Distillation Products Industries, Rochester, New York, 14603. About 50 ml. of the developing solvent was used in each case and the developing time depends upon the solvents as well as the absorbent but ranged from 1 - 5½ hours.

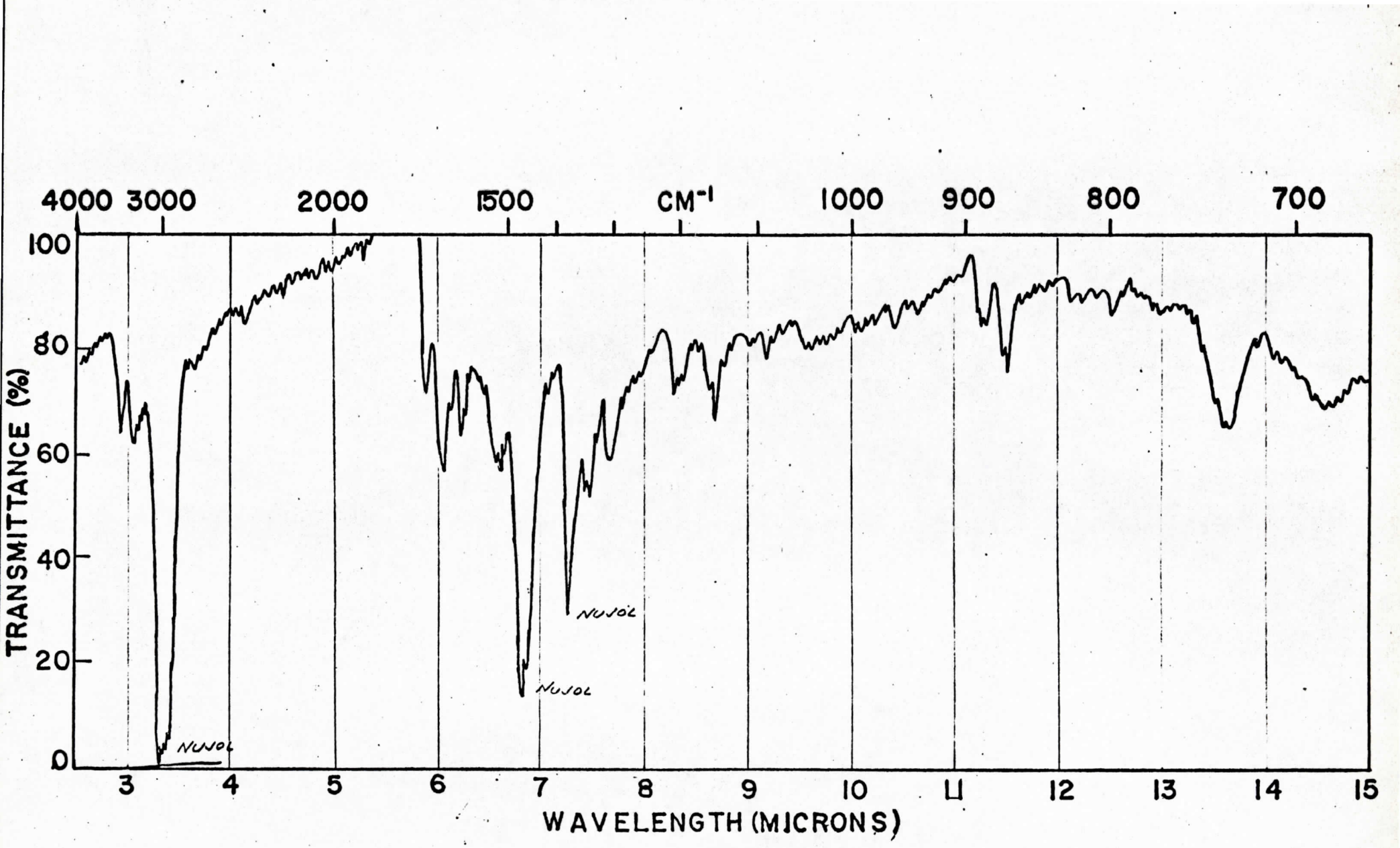
APPENDIX


4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
NUJOL
NUJOL
NUJOL
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

SPECTRUM I


4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
NUJOL
NUJOL
NUJOL
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

SPECTRUM 2


4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
NUJOL
NUJOL
NUJOL
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

SPECTRUM 3

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